

AD_____

Award Number: DAMD17-03-1-0387

TITLE: Training Program in Breast Cancer Prevention and Therapy
for Undergraduate Students

PRINCIPAL INVESTIGATOR: Rajendra G. Mehta, Ph.D.

CONTRACTING ORGANIZATION: University of Illinois
Chicago, Illinois 60612

REPORT DATE: May 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

20040907 079

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

| | | | | |
|---|---|--|---|--|
| 1. AGENCY USE ONLY (Leave blank) | | | 2. REPORT DATE May 2004 | 3. REPORT TYPE AND DATES COVERED Annual Summary (1 May 2003 - 30 Apr 2004) |
| 4. TITLE AND SUBTITLE Training Program in Breast Cancer Prevention and Therapy for Undergraduate Students | | | 5. FUNDING NUMBERS DAMD17-03-1-0387 | |
| 6. AUTHOR(S) Rajendra G. Mehta, Ph.D. | | | | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Illinois Chicago, Illinois 60612 <i>E-Mail:</i> raju@uic.edu | | | 8. PERFORMING ORGANIZATION REPORT NUMBER | |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012 | | | 10. SPONSORING / MONITORING AGENCY REPORT NUMBER | |
| 11. SUPPLEMENTARY NOTES | | | | |
| 12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited | | | | 12b. DISTRIBUTION CODE |
| 13. ABSTRACT (Maximum 200 Words) The overall objective of this training program is to provide an opportunity for undergraduate students to spend summer to get exposure to concepts of breast cancer research. We proposed that the majority of these students will be from a guaranteed professional program admission (GPPA) class. We proposed six students to be divided amongst 7-8 faculty members working closely in the area of breast cancer. During the last summer we had introduced the program to the Honors college and GPPA program office. As mentioned in the application we already had three GPPA students working in various laboratories who continued during the summer months and we admitted two more students to the summer training program. The sixth one had dropped out. Six mentors, each selected one student. The students learnt laboratory procedures and prepared a short report at the end of the summer. All faculty participants gave a short seminar on the concepts of their work as proposed. At the end of the summer the students discussed their accomplishments with the mentor and the PI. They all provided written overall evaluation of the program. | | | | |
| 14. SUBJECT TERMS Training, prevention, therapy, carcinogenesis | | | | 15. NUMBER OF PAGES 11 |
| 16. PRICE CODE | | | | |
| 17. SECURITY CLASSIFICATION OF REPORT Unclassified | 18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified | 19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified | 20. LIMITATION OF ABSTRACT Unlimited | |

Table of Contents

| | |
|--|-----------|
| Cover..... | 1 |
| SF 298..... | 2 |
| Table of Contents..... | 3 |
| Introduction..... | 4 |
| Body..... | 5 |
| Key Research Accomplishments..... | 6 |
| Reportable Outcomes..... | 10 |
| Conclusions..... | 11 |
| References..... | |
| Appendices..... | |

Award No: DAMD 17-03-1-0387

Title: Training Program in Breast Cancer Prevention and Therapy for undergraduate Students

PI: Rajendra G. Mehta, PhD
Professor, Department of Surgical Oncology
University of Illinois
Chicago, IL 60612-7317
Phone: (312) 413-1156
Fax: (312)996-9365
e-mail: raju@uic.edu

Dates: May 1, 2003 to April 30, 2004 (Summer Training Only)

INTRODUCTION:

The overall objective of this program is to provide training on the concepts and hands-on laboratory exposure to six undergraduate students who have outstanding credentials and are pursuing their BS degree at the University of Illinois with the intention of joining either medical school or further research career. The program is awarded for training during summer only.

The specific objectives of the the program were:

1. To establish a structured recruitment strategy for competent students in the second or third year of undergraduate degree program. Students who already have decided to be in the field of medical research or academic medicine will be given preference.
2. To provide an opportunity for them to spend summer in the laboratory of one of the mentors participating in this training program. The idea was for them to learn and conduct experiments in their respective laboratories under the supervision of the mentor.
3. To provide background on the subject of breast cancer biology, prevention and treatment, and experimental design to test the hypothesis. This is designed to provide an overview of the research in the laboratories of other mentors.
4. The students were required to prepare a written report and present their research in a seminar format at the end of summer training.

BODY OF THE REPORT

During the past summer we accomplished all the tasks originally proposed in our application. The intention was to identify six undergraduate students who are expected to gain training in the breast cancer area and hopefully continue their interest in conducting breast cancer research in future. This idea came up from one of our undergraduate students who was a GPPA student and worked in the PI's laboratory for one semester and then decided to continue doing research in addition to furthering her goals to join medical school. Currently Ms. Choi is registered for MD, PhD program with the intention of conducting PhD with Dr. Mehta. We intend to persuade students along these lines by providing them an overview of research approaches and the importance of it. It is by no means going to generate data of significant importance, since these students have never been exposed to laboratory research. Still we were pleasantly surprised to see the outcome of their summer efforts. Description from each student regarding their experience or research project during summer training is described in the application under accomplishments. Following is step by step description of the implementation of the training program.

Recruitment of students:

Since the program was awarded very close to the beginning of the summer, the program was not advertised in the University News Letter. However the PI contacted the appropriate personnel at the Honors College office and GPPA office for possible trainees. As a result we received several applications. From the pool of applications we selected four students. Two students were already conducting their research in various laboratories. Thus Pavan Jhaveri (Dr. Mehta Mentor) and Mr. Daniel Czys (Dr. Constantinou mentor) were already conducting studies and were transferred to the new program. Daniel will again continue his research this summer also.

Assignment of a mentor:

The students were provided with the names and contact information about the participating faculty members, which included Drs. Mehta, Das Gupta, Mehta RR, Constantinou, Christov, Diamond, Swanson and Salti. There were six students originally joined the program, but dr. Salti's student had dropped out within the first week of joining. Five students completed the training. The students in the program were:

Pavan Jhaveri (Junior, GPPA, Honors) Dr. RG Mehta Mentor
Daniel Czus (Junior Honors) Dr. Andreas Constantinou Mentor
Raj Shah (Junior, GPPA) Dr. Alan Diamond Mentor
Steven Wood (Senior, Honors) Dr. Konstantin Christov Mentor
Zhouhua Wang (Junior) Dr. Steve Swanson Mentor

Research Projects:

All students initiated their research with their respective mentors with the administrative responsibilities of Dr. RG Mehta (PI). The students attended a one hour presentation given by Dr. Mehta on the overall concept and the goals of the program. The rest of the time they spent their time in their respective laboratories. All students indicated that the program provided them with an overall idea about research in breast cancer. It gave them hands on experience with cell culture, cell counts, flow cytometry and PCR in some cases. Individual reports from each of these students in enclosed. Two students (Pavan and Daniel, who were also working prior to the program clearly have generated more results as compared to the first timers. However the entire document of their results is not included with this report.

Completion of training period:

All students prepared and submitted written reports, which are attached as an appendix. They all provided verbal evaluation of the program and indicated that they would be more interested in attending a formal 2-3 classes during the summer months to get an overview of the research in different laboratories. This was planned but was not implemented during the first year but will be carried out during the second and third year.

Tasks planned but could not complete:

- Advertisement ahead of time
- Students could not give a 20 minute presentation

In the second year these deficiencies will be corrected.

KEY RESEARCH ACCOMPLISHMENTS BY SUMMER STUDENTS:

Individual Student Reports:

Student 1

Project: Effects of Vitamin d analog in estrogen receptor negative breast cancer cells

Mentor: Rajendra Mehta, PhD

Student: Pavan Jhaveri (GPPA, Honors, Junior)

Report:

The active metabolite of vitamin D, 1 α ,25(OH)2D3 has been shown to induce cellular differentiation and/or apoptosis in several cancer cell lines. However, at the concentration needed for 1 α ,25(OH)2D3 to be effective, the metabolite induces hypercalcemia *in vivo*. As a result, various analogs of vitamin D3 have been developed. The majority of the

effective analogs still induce hypercalcemia. We identified an analog of vitamin D5, 1 α -hydroxy D5, which does not cause hypercalcemia at the concentrations needed to be effective and can be tolerated at a much higher concentration. The main mode of action reported for vitamin D is via binding to the vitamin D receptor (VDR) resulting in the regulation of key genes involved in differentiation and/or apoptosis. The purpose of the present study was to evaluate the effects of 1 α -hydroxy D5 on a breast cancer cell line, MDA-MB-231, reported to express very low levels of VDR. Although vitamin D5 had no effect on cell growth or differentiation as determined by flow cytometry and MTT assays, an upregulation of VDR was observed via Western Blot analysis. These results suggest that the VDR being expressed by the MDA-MB-231 cells may be truncated, polymorphic, or otherwise nonfunctional.

Although I was working in Dr. Mehta's laboratory for the past 6 months the additional summer training in the area of breast cancer research was extremely useful for my understanding of response to vitamin D in relation to steroid receptor status. If I am given a chance again, I probably would continue research in this area. I think this is an excellent program for Junior-Senior level undergraduates to appreciate scientific research and get some idea about what type of work is being carried out in breast cancer prevention.

Student 2

Project: The role of the essential trace element selenium in cancer prevention

Mentor: Alan Diamond, PhD

Student: Raj Shah

Report:

Raj became involved in a project investigating the role of the essential trace element selenium in cancer prevention. During his training period, he became familiar with the history of selenium and why there is a current focus on evaluating its chemopreventive properties. He was involved with two projects, both providing distinct laboratory experiences. One project involved the evaluation as to whether allelic variants of the selenium-containing protein glutathione peroxidase (GPx-1) respond differently to increasing amounts of selenium provided to the culture media. He became familiar with mammalian tissue culture practices and become proficient at the biochemical assay for GPx-1 activity. This assay included preparing cell lysates from breast cancer cells that were genetically engineered to exclusively express genetic variants of GPx-1 and exposed to varying amounts of selenium. GPx-1 activity was then determined from the lysates using a coupled spectrophotometric assay that measures the reduction in a chromophore as a direct consequence of the levels of GPx-1 in the extract. A second project Raj worked on was the development of a polymerase chain reaction (PCR)-based assay that quantifies the amount of DNA damage in plasmid DNA by measuring the consequential reduction in PCR amplification obtained using flanking primers. This approach is currently being used to assess whether selenium could reduce the level of damage in the test DNA sample. Collectively, this experience made Raj aware of the field of chemoprevention and exposed him to wide range of techniques and experimental approaches.

Student 3:

Project Title: Breast Cancer Research: Isoflavonic Phytoestrogens and Their Effect on Invitro Studies of Breast Cancer Cells.

Mentor: Andreas Constantinou, PhD

Student: Daniel Czyz

Report

In my opinion, when someone is appreciated and what is most important, given an opportunity to express his or her own ideas in the probable field of interest is the most important aspect of the research and further development of it. Luckily, I was given that opportunity by the research team in Dr. Constantinou's laboratory where my research was partially sponsored by the Department of Defense, which supported me financially, making me feel independent and exuberant. During the past few months I have been working with breast cancer cell lines analyzing their proliferation using MTT assay.

According to NABCO (National Alliance for Breast Cancer Organizations) one woman between the ages of 20 and 59 dies every 13 minutes in the United States alone, which accumulates to nearly 40,500 deaths per year. In the passed years these numbers were of much smaller magnitude; unfortunately, they tend to grow every year. These are very intimidating statistics; however, they can be lowered through a successful research.

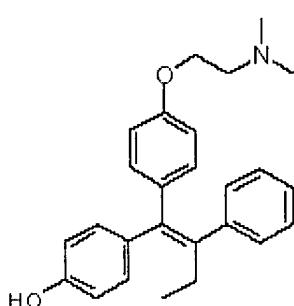
During the past few years breast cancer funding have multiplied tremendously (Breast Cancer Research Foundation). Many new organizations and programs arise to support the cancer research and involvement of the youngest scientists. One of these programs is sponsored by, as I mentioned above, the Department of Defense. The future of our loved ones, our families, our country, and of the world lies in the hands of those young and eager scientists. Such programs create a great opportunity and an invincible passion in those young peoples' minds, which leads them to dedication, affection to their work, and what is the most important-success.

Fortunately, I was lucky enough to receive support from Dr. Mehta from the Department of Defense for my breast cancer research. It partially changed my point of view and my estimations of the world of science. It brought me closer to my work and made me appreciate it more. Also, this opportunity strengthened my future goals in the cancer field.

During the summer of 2003, I was working with different breast cancer cell lines, those included MCF12F, a human non-tumorigenic, estrogen receptor negative immortal breast cell line and T47D:A18, which is also a human, but tumorigenic, estrogen receptor positive breast cancer cell line(1-2). I was given a task of analyzing the proliferation of these two cell lines where different isoflavones alone, or in combination with 4-hydroxy-tamoxifen,

were combined with the growing media. The effects of these treatments were analyzed by MTT Cell Proliferation Assay (Trevigen). It is a colorimetric assay based

measurement of cell proliferation and viability. MTT assay includes two chemicals,



yellow tetrazolium compound, which is reduced by metabolically active cells' mitochondria into purple formazan and a detergent that lyses cells and allows purple formazan to dissociate throughout each well of 96-well plate. When these two steps are done, the 96-well plate goes into a spectrophotometer where the absorbance is measured at 570nm wavelength (λ). The resulting absorbance is proportional to the number of cells according to the standard curve established prior to the experiment. My results showed that 4-hydroxy tamoxifen inhibited growth of estrogen receptor positive breast cancer cells.

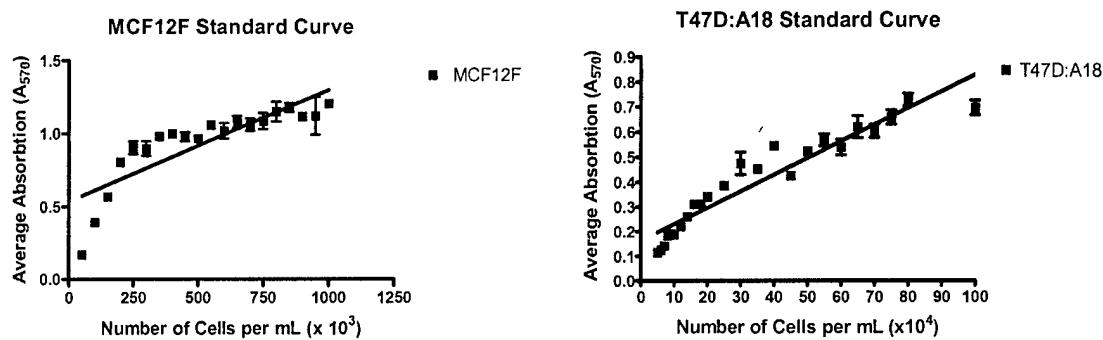


Figure 1. Shows two Standard Curves for MCF12F non carcinogenic cell line and T47D:A18 carcinogenic cell line. Both curves were established using MTT assay.

Student 4

Project title: Effects of Farnesyltransferase inhibitors (FTI) on breast tumor cells

Mentor: Konstantin Christov, MD, PhD

Student: Steven Wood...Junior Biology Major
Summer 2003

Report:

The main objective of my project was to learn several in vitro assays for estimation the efficacy of potential anti-tumor agents. As a model system, I use the MCF10A series of breast tumor cell lines: benign MCF10A, premalignant, MCF10AT, and malignant, MCF10CA1 cell lines. The latter two carry the H-Ras oncogen, which has been stable transfected. It is well known that FTI inhibit Ras protein synthesis and processing from the cytoplasm to the cell membrane, these agents should be predominantly efficacious in cells that carry the H-Ras oncogene.

I was involved in culturing the cells, in their treatment with different concentrations of FTI, R115777, an Janssen Pharmacia, Ltd. (Belgium) product, and in estimating their response to the FTI. As biomarkers of response I employed the: MTT assay, cell number alteration, cell cycle progression assay (flow cytometry data) and Image analysis of nuclear area. I found that FTI at 50nM suppressed by 50% the growth of cells transfected with H-Ras (MCF10AT and MCF10CA1 cell lines). The cells lacking H-Ras were resistant to R115777 and even 50 fold higher concentration was unable to bring their growth to the 50% level as compared to the control cells. By flow cytometry I found that FTI induced G2M arrest mostly in H-Ras transfected cells. By image analysis, I found that the arrested cells are with much higher nuclear area as compared to the control and non transfected cells.

I learned the basic principles of immunocytochemistry and how to identify nuclear and cytoplasmic antigens with monoclonal and polyclonal antibodies. I assessed the expression of ERK, AKT, p21 and p53 in subset of human and experimental tumors. I would like again to express my gratitude to the US Army Breast Cancer Summer Program for giving me the opportunity to be involved in studies related to breast cancer.

Student 5

Project Title: The role of cell receptor signaling and growth hormone and the effects it has on the occurrence and growth of breast cancer in rats and mice.

Mentor: Steven Swanson, PhD

Student: Zhouhua Wang

Report:

My position as a research assistant in Dr. Swanson's Medicinal Chemistry Laboratory has allowed me to develop my skills in cancer research, methods in molecular biology and animal work. I worked very closely with graduate assistant and aided her in trying to address role of cell receptor signaling and growth hormone and the effects it has on the occurrence and growth of breast cancer in rats and mice.

This research gave me the opportunity to learn and master the theory and execution of many common molecular protocols. My main responsibilities in this laboratory were to genotype the rats and mice to determine whether they possessed the SV40 cell receptor genotype and growth hormone genotype. To do so I was required to perform DNA extraction from a tail sample of the rat or mouse, optical density measures to determine the concentration of the DNA that was extracted, polymerase chain reactions to make multiple copies of the specific DNA sequence that characterizes each genotype, and gel electrophoresis to visualize the genotypes.

In addition to laboratory work I was also able to read research papers on current research on cancer in relation to cell signaling and growth receptors. Reading primary literature on cancer has not only exposed me to the most current findings and possible treatments for cancer but it has also allowed me to thinking critically about research methods and implications for results and applications. This research experience has been a great learning experience and will no doubt benefit me in the future in my graduate studies.

REPORTABLE OUTCOME

None

CONCLUSIONS:

During the first summer training program we were pleasantly surprised with the enthusiasm of students to learn the projects. We were disappointed by the number of applications we received even though the program was advertised in the GPPA program office and in the Honors College. The students learnt remarkably well. Mr. Cysz and Jhaveri plan to continue their involvement for the second year and these are the two students who had by far the best progress.

REFERENCES:

None

APPENDIX

None